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EXPERIMENTAL ARTICLES

Interaction of the Haloalkaliphilic Purple Bacteria *Rhodovulum steppense* with Aluminosilicate Minerals

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Abstract—The interaction was investigated between the haloalkaliphilic nonsulfur purple bacteria *Rhodovulum steppense* A-20s^T and layered aluminosilicates: micas (biotite, phlogopite, and muscovite) and clay minerals (montmorillonite and kaolinite). The interaction between all components of this system (minerals, water, medium, and bacteria) resulted in the changes in the chemical composition of the minerals and solutions. These changes were especially significant in the presence of bacteria. By using some elements for growth and promoting their transfer into the exchange pool of the minerals, bacteria removed these elements from the medium. The content of exchange bases in the aluminosilicates incubated in the presence of bacteria was several times higher than in the minerals incubated in sterile medium. The observed saturation of the mineral phase with potassium and magnesium may be considered the initial phase of diagenesis of the aluminosilicates under study.

Keywords: aluminosilicates, anoxygenic phototrophic bacteria, haloalkaliphiles, anaerobic conditions, exchange cations.

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Growing interest in the role of microorganisms in the transformation of rocks results from two major reasons. First, the scale of this interaction is global. The microbial biomass exceeds the total biomass of all other beings on Earth [1]. Microbial metabolism is highly versatile, so that microorganisms may thrive under practically any conditions occurring on this planet and transform the compounds of most of the elements. The second reason is associated with intense investigation of Archaean and Proterozoic rocks. The oldest rocks possibly bearing an indication of prokaryotic life are 3.8 Gyr old, only 0.2 Gyr after formation of the oldest known sedimentary rocks [2]. Bacteria seem to have been included in the geochemical cycles almost from the very beginning.

The theory of the interactions between living organisms and dead matter is presently at the stage of being formed, with intense accumulation of new data. Numerous investigations deal with the role of microorganisms in the genesis, transformation, and decomposition of minerals, and the number of such works is on the rise. Importantly, the experimental results are sometimes contradictory and vary greatly depending on the mineral, microbial species, and environmental conditions. However, analysis of the published data [3, 4] suggests a general conclusion that, while bacteria may carry out all the possible abiotic reactions associated with minerals, in the presence of microorganisms these reactions take place at higher rates and do not require specific conditions (high pressure, temperature, etc.), which are often needed under abiotic conditions. Thus, bacteria act as catalysts of geochemical processes.

The present work is part of an investigation of the interaction of bacteria with silicate minerals under anaerobic conditions [5]. Silicates and aluminosilicates comprise 95% of the mass of the Earth's crust. All igneous rocks, as well as many metamorphic and sedimentary rocks, consist mainly of minerals of this group. The layered aluminosilicates studied in the present work (micas and clay minerals) are present in all types of rocks. Their content in soil is significant, and they are to a large extent responsible for the chemical (buffer capacity, capacity for ion exchange, etc.) and physical (viscosity, water absorption, etc.) properties of soils [6]. Anaerobic haloalkaliphilic bacteria were chosen due to the scarcity of available data and the large scale of anaerobic biological weathering presently and especially in the geological past, when bacteria existed and free oxygen was absent. Moreover, modern concepts suggest that the inhabitants of soda lakes were probably among the first living organisms on our planet [7].

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Table 1. Growth of the phototrophic bacterium *Rhodovulum steppense* A-20s^T in the presence of aluminosilicate minerals

	Without minerals		With minerals under light				
	in the dark	under light	biotite	phlogopite	muscovite	montmorillonite	kaolinite
Protein concentration, mg/l	7*	250	242	248	258	240	234
Dry biomass weight, g/l	0.01	0.38	0.36	0.37	0.39	0.36	0.35
OD_{770}**	0.01	0.70	1.00	1.05	0.94	1.15	1.25

Notes: * All values are averages of four measurements.;

** Indicator of bacteriochlorophyll *a* content: optical density of the acetone extract from the cells in 5 ml of the cell suspension measured at 770 nm in a 0.5-cm cuvette.

MATERIALS AND METHODS

The haloalkaliphilic nonsulfur purple bacterium *Rhodovulum steppense* A-20s^T isolated from the Khilganta soda lake (Transbaikalia) [8, 9] was the microbiological subject of this work. The interaction of *Rdv. steppense* A-20s^T cultures with the layered aluminosilicate minerals: micas (biotite, phlogopite, and muscovite) and clay minerals (montmorillonite and kaolinite) was investigated. The mineral samples were obtained from the collection of the Vernadskii State Geological Museum, Russian Academy of Sciences. Bacteria were grown anaerobically under light with an organic electron donor and acceptor in the presence and absence of minerals. Aluminosilicates incubated with water or growth medium (without bacteria) were used as the controls.

The cultivation medium contained the following (g/l): KH_2PO_4, 0.33; NH_4Cl, 0.33; $MgCl_2 \cdot 6H_2O$, 0.33; KCl, 0.33; Na_2SO_4, 0.33; $NaHCO_3$, 5.0; NaCl, 10.0; and trace element solution, 1 ml/l. The trace element solution contained the following (g/l): EDTA, 5; $FeSO_4 \cdot 7H_2O$, 0.1 $ZnSO_4 \cdot 7H_2O$, 0.03 $MnCl_2$, 0.3 H_3BO_3, 0.2 $CoCl_2 \cdot 6H_2O$, 0.01 $CuCl_2$, 0.02 $NiCl_2 \cdot 2H_2O$, 0.02 $Na_2MoO_4 \cdot 2H_2O$, 0.02. Apart from the mineral compounds, the medium was supplemented with sodium acetate (2 g/l) and yeast extract (0.1 g/l); pH was adjusted to 7.8. Determination of the biomass content, of the chemical composition of the liquid phase, and of the content of exchange cations in the minerals, as well as X-ray diffraction analysis and microscopy, were carried out at the onset of the experiment and after 2.5 months of incubation.

Prior to inoculation the minerals (0.5 g) were sterilized for 20 min at 121°C in cultivation vials with a small amount of water. The cultures and controls were incubated under identical conditions: at 25–30°C under light (~2000 lx), in sealed 60-ml vials filled to capacity. The content of the vials was stirred twice a day. All experimental variants (in two repeats each) were incubated simultaneously.

Protein content in the cell suspension determined by the Lowry method [10] was measured to monitor the biomass growth. In order to exclude the possible effect of the minerals on this analysis, water containing the same mineral was used as the control. Calibration curves were developed for conversion of these data into other criteria of biomass growth (optical density of the cell suspension and dry weight of the biomass). The content of bacteriochlorophyll *a* was determined from the optical density of the acetone extract from the cells in 5 ml of the suspension. The optical density of the extract was measured on a KFK-3 spectrophotometer at 770 nm in a 0.5-cm cuvette.

Cell morphology was observed under an Olympus BX-41 phase contrast microscope.

The mineralogical composition of aluminosilicates was determined on a DRON-5 X-ray diffraction meter (Russia).

After sampling the remaining suspension was centrifuged and used for the chemical analyses of water and minerals. Chemical analysis was carried out as follows. The bulk elemental composition of aluminosilicate minerals was determined by X-ray fluorescence analysis. The composition of exchange cations of the minerals was analyzed by the Pfeffer method as modified by Molodtsov and Ignatova [11]. The concentrations of Fe, Ca, Mg, Mn, Cu, Ni, and Zn were determined by atomic absorption analysis on an AAS-3 spectrometer. Phosphorus was determined colorimetrically according to Murphy and Riley on a SPECOL-221 spectrophotometer [12]. Potassium and sodium were determined on an FLAFO-4 flame photometer [13].

RESULTS

Effect of Aluminosilicates on Bacterial Growth

The increase in bacterial biomass (measured as protein content) in the presence of all aluminosilicates studied was the same as in the control (Table 1). However, the average bacteriochlorophyll *a* content in the cultures grown with aluminosilicates was 1.5 times higher than in the control (Table 1). This was obviously the result of limited illumination due to the shading of bacteria by mineral particles. Since bacteria grown in the presence of aluminosilicates were under conditions of decreased illumination and had to synthesize additional photosynthetic pigments, equal illumination could have probably resulted in biomass

Table 2. Bulk elemental composition of investigated layered aluminosilicates

Macroelements, %						Microelements, μg/l					
	biotite	phlogopite	muscovite	montmorillonite	kaolinite		biotite	phlogopite	muscovite	montmorillonite	kaolinite
MgO	2.467	22.795	0.091	3.363	0.297	Ni	43	25	11	14	17
Al_2O_3	10.609	13.217	38.306	17.075	39.478	Cu	31	12	9	32	26
SiO_2	37.119	39.930	45.998	63.620	47.838	Zn	1322	76	44	100	33
P_2O_5	0.037	0.131	0.000	0.082	0.007	Ga	57	11	56	27	54
SO_3	0.092	0.056	0.027	0.224	0.020	As	0	0	0	0	17
Cl	0.056	0.225	0.052	0.064	0.009	Br	0	0	0	0	0
K_2O	13.846	12.654	11.579	1.065	1.491	Pb	13	12	11	31	38
CaO	0.037	0.123	0.033	3.979	0.051	Rb	646	593	237	38	200
TiO_2	3.397	0.963	0.809	0.759	0.118	Sr	38	43	45	464	78
Cr_2O_3	0.018	0.002	0.002	0.002	0.004	Y	15	12	9	36	31
MnO_2	0.873	0.079	0.021	0.073	0.020	Zr	16	15	7	287	133
Fe_2O_3	26.094	6.099	1.974	3.065	0.672	Nb	232	50	48	17	10

growth higher than in the control. Thus, the aluminosilicates studied had probably a positive effect on bacterial growth.

The cells of *Rhodovulum steppense* A-20s grown with and without aluminosilicates had a similar morphology typical of this strain; i.e., they were straight rods of 0.5–0.7 × 0.8–1.4 μm. Bacteria grown in the media with clay minerals (montmorillonite and kaolinite) had, however, a somewhat more elongated shape.

Changes in Chemical Composition of Solutions

Throughout the experiment X-ray diffraction analysis did not reveal drastic changes in the mineralogical composition of aluminosilicates incubated with and without bacteria. Importantly, however, this method has low sensitivity. Only formation of new minerals in significant amounts results in reliable changes in the X-ray diffraction patterns. At the same time, analytical methods revealed significant changes in the chemical composition of aluminosilicates and the ambient solutions.

It should be noted that significant differences both in the chemical composition (Table 2) and the crystal lattice structure existed between the layered aluminosilicates used in the present work. Apart from the general patterns of their interaction with solutions and bacteria, peculiarities specific for the individual minerals were therefore observed (Tables 3, 4).

Interaction with water (Table 3) resulted in release of 1–7 mg/l potassium and of small amounts (<1 mg/l) magnesium and calcium from all minerals. Iron was eluted only from some minerals: about 2 mg/l was found in biotite-containing water a 0.35 mg/l, in the water with phlogopite. Biotite contained the highest amount of iron (26%, Table 2). Elution of a small amount of phosphorus (0.7 mg/l) was observed only in the case of montmorillonite.

Interaction between minerals and the medium (Table 3) may be assessed as the $MMi–MB_D$ value, i.e., the difference between the concentrations of specific elements in the sterile medium with minerals and in the dark control (inoculated medium without minerals, which was incubated in the dark, without cell growth and related changes in the composition of the medium). For all minerals a certain increase of calcium in the medium was observed (by 1.6 mg/l for montmorillonite and by 0.1–0.3 mg/l for other minerals). Potassium concentration in the sterile medium increased significantly (by 20 mg/l) only in the presence of biotite and phlogopite and to a lesser degree (by 5 mg/l) in the presence of kaolinite. Magnesium concentration increased significantly (by 11 mg/l) only in the medium with montmorillonite. In all other cases, the effect of aluminosilicates on the chemical composition of the medium was insignificant. The rate of solubilization of an element did not always correlate with its content in the mineral (Tables 2, 3) to a greater degree depending on the structural features of aluminosilicates. For example, the highest mobility of the elements was found in montmorillonite, which has a loose crystal lattice.

The interaction between bacteria and the medium (Table 3) was assessed as the difference between the elemental concentrations in inoculated media without minerals incubated in the dark and under light ($MB_L–MB_D$), while ***the interaction between bacteria and the mineral–medium system*** was assessed by the difference between inoculated and sterile mineral-containing media (MMiB–MMi). In the course of growth both with and without aluminosilicates, bacteria con-

Table 3. Concentration of the chemical elements in solutions, mg/l

	Mineral	MiW*	MiM	MiMB	MB_L	MB_D	$MiM-MB_D$	MiMB–MiM	MB_L-MB_D	(MiMB–MiM)–(MB_L-MB_D)
Phosphorus	Without minerals				60.16**	77.34			**–17.2**	
	Biotite	0.02	76.91	60.52			**–0.4**	**–16.4**		**–0.8**
	Phlogopite	0.03	77.78	61.21			**+ 0.4**	**–16.6**		**–0.6**
	Muscovite	0.00	76.91	59.47			**–0.4**	**–17.4**		**+0.2**
	Montmorillonite	0.66	77.17	59.91			**–0.2**	**–17.3**		**+0.1**
	Kaolinite	0.00	76.90	61.02			**–0.4**	**–15.9**		**–1.3**
Potassium	Without minerals				262.50	272.50			**–10.0**	
	Biotite	6.68	297.50	260.00			**+25.0**	**–37.5**		**–27.5**
	Phlogopite	5.40	290.00	262.50			**+17.5**	**–27.5**		**–17.5**
	Muscovite	2.93	272.00	258.50			**–0.5**	**–14.5**		**–4.5**
	Montmorillonite	4.83	271.00	231.50			**–1.5**	**–39.5**		**–29.5**
	Kaolinite	0.62	277.50	258.00			**+5.0**	**–19.5**		**–9.5**
Magnesium	Without minerals				37.25	44.35			**–7.1**	
	Biotite	0.78	43.50	33.00			**–0.9**	**–10.5**		**–3.4**
	Phlogopite	0.40	44.65	32.00			**+0.3**	**–12.7**		**–5.6**
	Muscovite	0.25	43.17	32.25			**–1.2**	**–10.9**		**–3.8**
	Montmorillonite	0.17	55.27	23.00			**+11.0**	**–32.3**		**–25.2**
	Kaolinite	0.44	44.42	33.50			**+0.1**	**–10.9**		**–3.8**
Calcium	Without minerals				0.76	0.81				
	Biotite	0.37	1.00	1.35			**+0.2**	**+0.4**		**+0.4**
	Phlogopite	0.88	1.15	1.52			**+0.3**	**+0.4**		**+0.4**
	Muscovite	0.39	0.91	1.18			**+0.1**	**+0.3**		**+0.3**
	Montmorillonite	0.17	2.43	4.29			**+1.6**	**+1.9**		**+1.9**
	Kaolinite	0.34	0.98	1.38			**+0.2**	**+0.4**		**+0.4**
Iron	Without minerals				0.11	0.30			**–0.2**	
	Biotite	1.88	0.30	0.12			**0**	**–0.2**		**0.0**
	Phlogopite	0.35	0.33	0.08			**0**	**–0.3**		**–0.1**
	Muscovite	0.03	0.29	0.12			**0**	**–0.2**		**0.0**
	Montmorillonite	2.18	0.31	0.07			**0**	**–0.2**		**0.0**
	Kaolinite	0.02	0.29	0.10			**0**	**–0.2**		**0.0**

Notes: * Mi, mineral; W, water; M, medium, B, bacteria; L, under light; D, in the dark. Results of the interactions: $MiM-MB_D$, between the mineral and the medium; MiMB–MiM, between the mineral + medium system and bacteria; MB_L-MB_D, between bacteria and the medium.;

** All values are averages of four measurements.

Table 4. Content of exchange cations in the layered aluminosilicates, mg-eq/100 g

	Mineral	MiW	MiM	MiMB	Effect of		
					medium, MiM–MiW	bacteria, MiMB–MiM	medium and bacteria, MiMB–MiW
Sodium	Biotite	0.31	0.81	3.39	**+0.5**	**+2.6**	**+3.1**
	Phlogopite	0.11	0.54	3.50	**+0.4**	**+3.0**	**+3.4**
	Muscovite	0.19	0.39	3.10	**+0.2**	**+2.7**	**+2.9**
	Montmorillonite	12.52	14.47	70.19	**+2.0**	**+55.7**	**+57.7**
	Kaolinite	0.61	1.70	4.79	**+1.1**	**+3.1**	**+4.2**
Potassium	Biotite	0.26	0.88	1.12	**+0.6**	**+0.2**	**+0.9**
	Phlogopite	0.34	0.38	0.87	**0**	**+0.5**	**+0.5**
	Muscovite	0.22	0.36	0.76	**+0.1**	**+0.4**	**+0.5**
	Montmorillonite	1.22	6.94	9.48	**+5.7**	**+2.5**	**+8.3**
	Kaolinite	0.63	0.62	1.02	**0**	**+0.4**	**+0.4**
Magnesium	Biotite	0.71	0.97	3.15	**+0.3**	**+2.2**	**+2.5**
	Phlogopite	0.25	0.88	4.07	**+0.6**	**+3.2**	**+3.8**
	Muscovite	0.00	0.88	3.22	**+0.9**	**+2.3**	**+3.2**
	Montmorillonite	2.81	4.82	10.97	**+2.0**	**+6.2**	**+8.2**
	Kaolinite	0.72	0.69	2.92	**0**	**+2.2**	**+2.2**
Calcium	Biotite	0.42	0.00	0.30	**–0.4**	**+0.3**	**–0.1**
	Phlogopite	1.42	0.52	0.56	**–0.9**	**0**	**–0.9**
	Muscovite	0.19	0.00	0.00	**–0.2**	**0**	**–0.2**
	Montmorillonite	3.64	1.50	2.91	**–2.1**	**+1.4**	**–0.7**
	Kaolinite	0.81	0.00	0.00	**–0.8**	**0**	**–0.8**
Total	Biotite	1.70	2.66	7.96	**+1.0**	**+5.3**	**+6.3**
	Phlogopite	2.12	2.32	9.00	**+0.2**	**+6.7**	**+6.9**
	Muscovite	0.60	1.63	7.08	**+1.0**	**+5.5**	**+6.5**
	Montmorillonite	20.19	27.73	93.55	**+7.5**	**+65.8**	**+73.4**
	Kaolinite	2.77	3.01	8.73	**+0.2**	**+5.7**	**+5.9**

Note: all designations are as in Table 3.

sumed phosphorus, potassium, magnesium, and some iron from the medium. In the presence of bacteria, calcium concentration remained almost the same in the medium without minerals and increased slightly (by 0.3–2 mg/l) in the media with aluminosilicates. The concentrations of dissolved nickel, manganese, zinc, copper, cobalt, and molybdenum either did not change in any of the experimental variants, or the change was below the sensitivity level of the analytical methods applied.

Importantly, the changes in the concentration of some ions were different in inoculated media with and without minerals (Table 3). For example, in the presence of aluminosilicates, the decrease in magnesium and potassium concentrations in inoculated media (compared to the sterile one, MMiB–MMi) was 1.5–4.5 times higher than their consumption by bacteria from the media without minerals (MB_L–MB_D). For different aluminosilicates this additional decrease of the concentration [(MMiB–MMi)–(MB_L–MB_D)] was from 5 to 30 mg/l for potassium and from 3 to 25 mg/l for magnesium. Since the biomass growth was approximately the same with and without minerals, some of the potassium and magnesium ions released from the medium evidently increased the pool of exchange bases in aluminosilicates. Moreover, a certain increase in magnesium consumption is probably associated with additional bacteriochlorophyll *a* synthesis in the presence of minerals.

Changes in Composition of Exchange Cations in Aluminosilicates

The concentration of exchange bases (sodium, potassium, magnesium, and calcium ions) was determined in all mineral samples incubated under various

experimental conditions. Significant changes were found in the composition of exchange cations after the experiment (Table 4).

The changes in the ***total content of exchange bases*** were most pronounced in the presence of bacteria. Incubation of the minerals in sterile medium resulted in a slight increase of the pool of exchange bases (by 0.2–7.5 mg-eq/100 g). More significant changes occurred in the aluminosilicate samples incubated with inoculated medium. In the presence of bacteria, the total content of exchange bases increased greatly in all minerals. This increase was 66 mg-eq/100 g for montmorillonite and 5–7 mg-eq/100 g for other minerals. Thus, the simultaneous effect of the medium and bacteria resulted in an increase of the total exchange bases. While in micas and kaolinite this increase was approximately the same (6–7 mg-eq/100 g), in montmorillonite it was much higher (73 mg-eq/100 g).

The ratio of exchange bases also changes during the experiment (Table 4). In the presence of the medium, the content of sodium (in all samples), potassium (in all samples except for phlogopite and kaolinite), and magnesium (in all samples except for kaolinite) increased somewhat. This increase was 2–6 mg-eq/100 g for montmorillonite and less than 1 mg-eq/100 g for other aluminosilicates. On the contrary, the content of exchange calcium in the presence of the medium was lower than in minerals incubated with water. This decrease was 2.1 mg-eq/100 g for montmorillonite and less than 1 mg-eq/100 g for other minerals.

In the presence of bacteria, the changes were much more significant. Bacteria promoted the process of saturation of the minerals with bases. The increase in the share of sodium, potassium, and magnesium in the exchange pool of the aluminosilicates was usually several times higher in the presence of bacteria than in the minerals incubated in the sterile medium. Incubation of the minerals in inoculated media resulted in an increase in the content of each of these cations by 3–55 mg-eq/100 g for montmorillonite and by 0.2–3 mg-eq/100 g for other minerals. In the presence of bacteria, the share of exchange calcium either did not change or increased somewhat, although it remained below the level observed in the mineral + water variant. Simultaneous action of the medium and bacteria resulted therefore in a certain decrease (by 0.1–0.8 mg-eq/100 g) in the calcium exchange pool compared to its initial value (MW).

DISCUSSION

Thus, the interaction of all components (minerals, water, medium, and bacteria) in the multicomponent systems of the experimental variants was shown (Tables 2–4). These interactions resulted in changes in the chemical composition of both minerals and solutions. In the presence of bacteria, the changes were especially pronounced. Bacteria consumed some elements of the medium for cell growth and promoted their transfer to the exchange pool of the minerals. In aluminosilicates incubated with bacteria, the content of exchange bases was several times higher than in the minerals incubated in the sterile medium. This effect may be partially due to the pH increase in the course of bacterial growth (on average by 0.5 pH units). However, this alkalization is insufficient to explain the significant changes in the chemical composition of the minerals. Local alkalization around the cells that lay in close contact with the minerals may play a certain role in the saturation of aluminosilicates with bases. Other mechanisms are possible and require special investigation. The investigated layered aluminosilicates had, in turn, a stimulatory effect on bacterial development. While the biomass growth was the same, in the presence of minerals, 1.5 times more bacteriochlorophyll *a* was synthesized by the cells.

We reported similar results earlier for the interaction of *Rdv. steppense* A-20s^T with volcanic ash [5]. Similarly to the present experiment, bacteria promoted the saturation of ash samples with bases, although the cation ratio was different due to the different mineralogical composition of volcanic ash.

The differences between aluminosilicates in their interaction with solutions and bacteria are due to the differences in their chemical composition and the crystal lattice structure. For example, the most pronounced changes were observed for montmorillonite, which has the highest cation exchange capacity (60–150 mg-eq/100 g) due to a significant crystal lattice interlayer space. For comparison, in the case of kaolinites, the cation exchange capacity varies from 3 to 15–20 mg-eq/100 g [6].

Our results indicate possible geological consequences of the interaction between minerals and microorganisms. The observed changes in the chemical composition of aluminosilicates indicate that new minerals may be formed under varied experimental duration and conditions. The probability and rate of the transformation of minerals increase significantly in the presence of bacteria. In the present experiment, layered aluminosilicates were saturated with potassium, sodium, and magnesium cations. Saturation of montmorillonite with magnesium may result in formation of chlorite, chlorite–smectite, or saponite, while saturation with potassium may lead to formation of illite or muscovite. Evidence exists of chlorite formation from montmorillonite in the presence of magnesium-enriched seawater, while illite is the most widespread clay mineral in marine sediments [6]. Generally, in the process of diagenesis illite and chlorite are formed and montmorillonite and kaolinite disappear, so that slates and mudstones contain mostly the former two minerals. Thus, the saturation of the mineral phase with potassium and magnesium observed in the present experiment may be considered as the initial stage of diagenesis of the aluminosilicates in question. The rate of transformation of these minerals increased drastically in the presence of bacteria.

These data suggest the probable involvement of anaerobic microflora in the transformation of aluminosilicates, especially in the case of their contact with highly mineralized solutions with elevated concentrations of potassium and magnesium.

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